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Transgenic Bt Corn: Is This the Future of Insect Pest Management?

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Introduction

Marlin E. Rice

In a few short years, it is expected that a dramatically different management tactic will be ready for field corn producers to combat the ravages of the European corn borer. This new tactic currently goes by a variety of names including genetically-engineered corn, transgenic corn, *Bt* corn, or transgenic *Bt* corn. But whatever name eventually is used, it offers a hope that biotechnology will deliver a corn plant that is resistant to leaf feeding, stalk tunneling, dropped ears and reduced grain yields caused by the European corn borer.

This presentation will be a blend of five different research reports conducted by both industry and university researchers. These reports will provide some insight into what transgenic *Bt* corn is and how it was developed, what pest and beneficial insects it does or does not affect, and how both transformed and nontransformed corn plants respond to insect feeding or the absence thereof. Although the title asks if transgenic *Bt* corn is the future of insect pest management, we will not be able to answer that question at this time. Only the farmers of the Midwest will determine whether transgenic *Bt* corn is embraced or rejected as a management tactic for the European corn borer.

Part I

Bt TRANSGENIC CORN

Mark Pieper

Ciba is a worldwide biological and chemical group, dedicated to satisfying our customers' needs in industry, health care, and agriculture with innovative products and services. Ciba Seeds business is focused on the development and application of new technologies. The development of these technologies for agriculture requires concentrated research and extensive testing. This will bring new hybrids to the market earlier. In 1983, we furthered our commitment to research by opening our Agricultural Biotechnology Research Unit (ABRU) in Research Triangle Park, North Carolina, and expanded our conventional research program with the addition of eight stations across the cornbelt. Ciba's Agricultural Biotechnology Research Unit (ABRU) forms the backbone of our new technologies research. The research generated by our scientists is used to produce new traits in hybrids that will benefit agriculture.

Recombinant DNA technology is one of the tools used to generate improved hybrids. This technology involves moving a gene from one species to another. The gene may be moved between or within different species. Recombinant DNA technology is a viable way for us, at Ciba Seeds, to initiate the expression of desirable characteristics in corn hybrids. Recombinant DNA is defined as new associations of DNA sequences assembled in the laboratory from the same or different organisms. This technology includes all the techniques involved in the construction, study, and use of recombinant DNA molecules as well as gene discovery, gene modification and transformation.

The first step to create a new product, such as Bt corn, through recombinant DNA technology is finding a specific gene that expresses a desirable characteristic. In an on-going research effort, our scientists are conducting extensive searches to discover and isolate genes that provide desirable traits.

The protein that makes our Bt corn resistant to European Corn borer was found in a bacterium Bacillus thuringiensis, which has been use commercially as a spray-on for over 30 years to control various types of insects. Bt is a powerful means of biological control because all stages of European corn borer larvae are susceptible.

After scientists identify and characterize an appropriate protein, the next step is to design a gene that will successfully express that protein in the desired plant species. A number of adjustments were required to get the Bt protein to express itself in a desirable way in the corn plant.

Once scientists find or build a high-value gene, they are ready to insert the gene into a corn cell. This is the process of transformation. Our Bt corn is a transformed product. There are several different methods that have been used for the transformation of genes into plant cells. The tool that has been used to insert a gene into a corn cell with greatest success to date has been the "gene gun". Using this method, tiny particles coded with the new gene are propelled by a burst of helium gas through the cell wall.

Cells that have been treated with a gene gun are then tested to see whether or not the genes have been received and incorporated into the plant chromosome. In the test, called a bioassay, the cells that have been transformed change color. Transformed cells are then propagated by tissue culture into small seedlings. These seedlings are grown into whole plants in trays referred to as "plant condos". The first plants that result from transformation and tissue culture propagation are grown to full maturity in our research greenhouses to see if they retain the characteristic of the new gene. Seeds produced from the tested plants are collected and grown to see if the plants have the ability to pass the characteristic on to future generations. If so, the plants have been "transformed" successfully and now possess the desired gene.

The transformed plants then undergo further testing in the greenhouse and in the field to find the level of effectiveness of the characteristic. The new trait can then be bred into commercial lines through our traditional breeding methods, aided by the use of RFLP'S. The hybrids will then undergo rigorous field testing before being introduced into the marketplace.

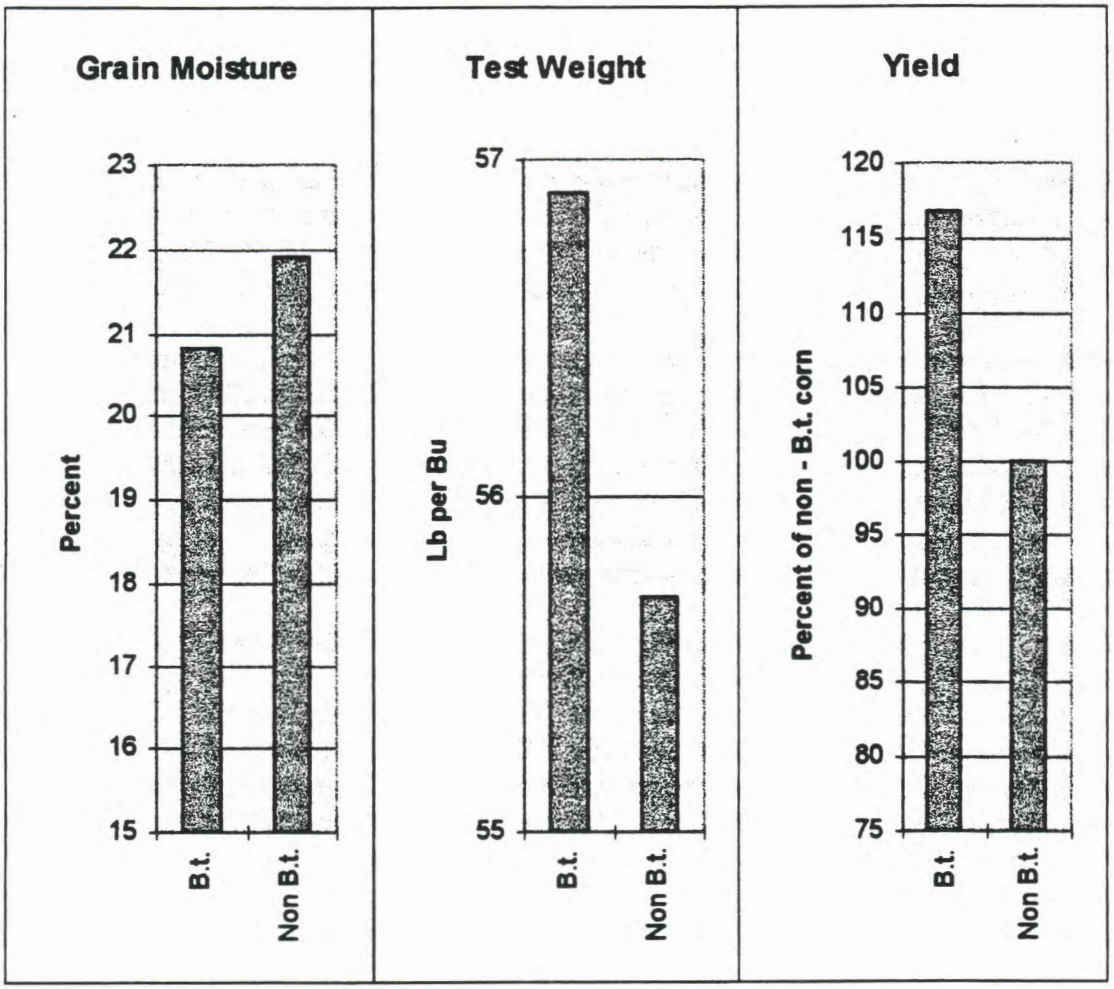
An indispensable part of the product development process at Ciba Seeds is to secure the approval of all appropriate regulatory agencies, such as the USDA, EPA, FDA and state authorities. The Environmental Protection Agency granted Ciba Seeds the first Experimental Use Permit for large-scale field trials with transgenic corn in July of 1993. In July of 1994, Ciba Seeds submitted to the Environmental Protection Agency an application for registration of this genetically engineered corn. With this application Ciba Seeds became the first company to seek full federal approval of a staple grain crop that was produced through DNA technology.

The protein present in the Bt corn works similar to the active protein contained in the bio-insecticidial, Bt's currently on the market today. When a European corn borer larvae consumes part of the plant containing Bt., a protein interacts with the alkaline gut of the larvae, forming a substance which causes the insect to stop feeding. The activated protein binds specifically to receptors in the midgut of the European corn borer and breaks down cells causing the formation of pores. The protein in the Bt corn is expressed predominately in the green tissue and pollen of the plant, with lessor concentrations in the pith, roots, and kernels. The expression of the protein is highest during the vegetative and reproductive stages of the plant. As the plant starts to senesce, the protein levels start to decrease.

In 1994, Ciba Seeds field tested, in over 70 locations throughout the corn belt, corn containing a gene from "Bt". Sixteen of these trials were located across Nebraska and the Western Plains. Throughout the growing season, data were collected on plants that were both naturally and artificially infested with corn borer. Positive results have been observed in reducing yield losses due to European corn borer in Bt corn versus non Bt corn developed from the same inbred lines. These results include more harvestable grain, increased standability, increased test weight and faster drydown (Figure 1).

Ciba Seeds is committed to bringing products to the marketplace that provide a high level of performance and that are safe for consumers and the environment as well. Transgenic plants are expected to become a valuable biological component of a system of integrated pest management. Biotechnology may provide alternative management strategies that will improve the efficiency and productivity of agriculture.

Figure 1. Bt corn verses non-Bt corn comparisons in twelve locations across Nebraska. These plots were naturally infested with European corn borer. The number of tunnels caused by the borer in the non-Bt corn ranging from .04 to 5.2 tunnels per plant, and averaged 3.8 tunnels per plant.



Part II

RESPONSES OF FIVE CATERPILLAR SPECIES FEEDING ON TRANSGENIC *BT* CORN

Marlin E. Rice and Clinton D. Pilcher

Corn in the Midwest is annually attacked by a variety of caterpillar species including the European corn borer, black cutworm, corn earworm, armyworm, and stalk borer. The European corn borer is the most destructive of these five species, and it could be argued that it also is the number one corn pest in Iowa. Yield losses from just the second generation of the European corn borer have recently been as high as 33 bushels per acre in field corn (Rice 1991). Crop injury caused by the other four species is seldom this substantial, but yield losses can occur. Black cutworms are perceived as annual threats to seedling corn and during some years thousands of acres are sprayed with insecticides to prevent stand loss. Corn earworms will damage the kernels in ears of late-planted corn. Stalk borers can kill or stunt plants adjacent to grass terraces, waterways, or where annual and perennial grass control has been poor in the field. Armyworms can strip young plants when the corn is no-tilled into pasture, rye, or after they move from herbicide-killed grasses within the field.

Management options for control of these insects are relatively few and fall into two categories - prevention and cure. Examples of preventive tactics that would prevent or reduce crop damage include weed management in the spring prior to immigration or egg laying (black cutworms); control of annual and perennial grasses in the late summer to discourage egg laying (stalk borers); early planting to avoid later- season injury (armyworms, black cutworms, European corn borer, corn earworm); and planting hybrids with low to moderate levels of resistance (European corn borer). Examples of curative tactics are insecticides. Insecticides are commonly used at planting, or later when seedling cutting occurs, to prevent damage from the black cutworm. They are occasionally used to control first generation European corn borers. Rarely are they used to manage second generation European corn borers and corn earworms.

Most insecticides are synthetic compounds with a broad spectrum of activity against both pests and beneficial insects. One exception is the microbial insecticide (also called a biological insecticide) that contains *Bacillus thuringiensis* (hereafter abbreviated *Bt*).

Bt is a naturally-occurring bacterium found in the soil and on leaves. It is deadly to many insect species including many Lepidoptera (moth caterpillars), Diptera (fly larvae), and Coleoptera (larvae and adult beetles). It is harmless to humans, other mammals, birds, fish and beneficial insects. Many chemical companies have formulated *Bt* into commercial products; one of the most common being DiPel. *Bt* has found greater acceptance with some seedcorn companies because of its relative nontoxicity to humans and less restrictive field reentry intervals. Both of these are attributes when laborers must be in the field.

An insect must eat *Bt* before it can be killed. Here is briefly how it works. The insect eats the *Bt* which is a combination of spores and crystals. If the insect has a gut with a high pH (alkaline), then the crystals dissolve and produce toxins that perforate the lining of the midgut. The insect stops feeding shortly after ingesting a lethal dose of *Bt* and does not feed again. The spores

then pass through the perforations of the midgut, entering the blood system and body cavity. Here the spores germinate and begin to reproduce within the insect. The insect often dies within three to four days.

In recent years, several agricultural companies have pursued an alternative method of insect control - genetically-engineered plants with resistance to feeding by insects. One company, Ciba Seeds, has inserted a synthetic, truncated version of the *Bt* gene, known as *cryIA(b)*, into corn plants. This truncated gene produces the same insecticidal toxin as the full-length protoxin once it is processed inside the insect gut (Koziel et al. 1993). These genes were introduced into corn embryos using a system in the laboratory known as mircroprojectile bombardment. Plants that contained the *Bt* gene (transgenic) and the same hybrid without the gene (iosgenic) were later transferred to the field by Ciba and tested against European corn borer larvae. Each plant was inoculated with 300 newly-hatched larvae per week, beginning at the mid-whorl stage and continuing for eight weeks.

Results of the Ciba field trials indicated that less leaf-feeding injury occurred on the transgenic *Bt* corn, the average tunnel length from the second generation larvae was reduced by 79-97 percent, and mortality of larvae fed leaf pieces from transgenic Bt plants grown in the field was 55-100 percent (Koziel et al. 1993).

This year we examined a transgenic *Bt* corn hybrid and looked at both the responses of European corn borer, black cutworm, stalk borer, armyworm and corn earworm on the plant. Here we will present some of the preliminary results of this study.

Materials and Methods

Ciba Seeds hybrids 3906BtX (transgenic) and 3906X (isogenic) were planted by hand at the rate of 26,100 plants per acre on the Iowa State University campus on May 2. Seeds were planted in four replications consisting of four rows of each hybrid. Rows two and three in each treatment were used for all experiments except for the European corn borer, where rows one and four were used. Fertility and weed control were provided by Farm Services, ISU, and followed recommendations for field corn production in central Iowa.

Black cutworm. Larvae were placed on three-leaf corn and allowed to feed on the plants. Two categories of larvae were used: leaf-feeding stage larvae (first and second instars) and stalk-cutting stage larvae (fourth and fifth instars). These two categories of larvae were not mixed on the same plant. Each corn plant was enclosed in an open-ended one gallon can. Twenty plants were evaluated in each replication for a total of 80 plants for each genotype and each larval category. Plants were evaluated approximately every other day for leaf feeding and stalk cutting. The following rating scale was used for leaf-feeding larvae: 1 = no leaf feeding or cutting; 2 = light skeletonizing with leaf feeding on less than 1/2 the leaf; 3 = heavy skeletonizing with leaf feeding on more than 1/2 the leaf; 4 = leaf cut or plant dead. A slightly different scale was used for plant-cutting larvae: 1 = no feeding or cutting; 2 = leaf feeding or skeletonizing; 3 = one leaf cut; 4 = two or more leaves cut; 5 = stalk cut above ground; and 6 = stalk cut below ground.

Stalk borer. Third and fourth stage larvae were collected from brome grass in May. A single larva was placed in the whorl of a four-leaf stage plant on May 31. Each plant was enclosed in

an open-ended five gallon plastic bucket. Eighty plants of each genotype were infested. Plants were evaluated for leaf feeding for two weeks. The following rating scale was used: 1 = plant infested or minor leaf feeding; 2 = minor leaf feeding, plant tunneled, growing point not injured; 3 = heavy leaf feeding, growing point not injured; and 4 = dead heart with growing point killed. Stalks were split in October and the length of tunneling was measured.

Armyworm. Newly-hatched larvae were inoculated with a mixture of corn grits into the whorl of seven-leaf stage plants on June 15. Approximately 15 larvae were placed on each plant. Eighty plants in each treatment were infested. Plants were evaluated for leaf feeding 13 days after infestation. The number of feeding scars > 2 cm and < 2 cm were counted on each plant.

Corn earworm. Five first-stage larvae were placed in the silks of 80 plants in each genotype. Each ear was evaluated 15 days later and the number of live larvae was recorded.

European corn borer. Recently-hatched larvae were inoculated with a mixture of corn grits onto the plants. Infestations consisted of approximately 75-100 larvae per plant for eight weeks, beginning with the eight-leaf stage (mid June) and continuing through the 18-leaf stage (second week of August). Eighty plants were infested in each genotype. Stalks were split in October and the length of tunneling was measured, live borers were counted, and the number of tunnels per ear shank were recorded. The grain from each infested plant was harvested, weighed and adjusted to 15.5% moisture.

Each of these five caterpillar species was also tested in the laboratory. Recently-hatched larvae were placed in individual vials and fed pieces of leaf tissue from newly-emerging whorl leaves. Larvae were fed leaf tissue for 48 hours and then transferred to an artificial diet. Larvae for each experiment were exposed to the same temperatures, humidity, and light:dark phases. Data that were recorded included larval mortality, weight of pupae that survived to this stage, and days to moth emergence.

Results and Discussion

A summary of the preliminary results for all five species is shown in Table 1.

Black cutworm. There was no difference in the amount of leaf feeding or stalk cutting in either the isogenic or transgenic *Bt* corn. Larval mortality and pupal weights were similar when larvae feed on either genotype. These results are not surprising since commercial formulations of *Bt* have long been considered ineffective against this insect.

Stalk borer. The average leaf rating was less in the transgenic corn with the *Bt* than the non*Bt* corn. The average tunnel length was very small in both genotypes and suggests that there were no differences. Mortality of larvae was slightly higher for insects that fed on transgenic Bt corn, but of those that survived there were no differences in pupal weight or days to moth emergence.

Armyworm. The amount of feeding injury was minor on both genotypes, but there did appear to be differences in the amount of feeding scars less than 2 cm in length. The transgenic *Bt* corn had 36 percent fewer feeding scars than the isogenic non*Bt* corn. Armyworms were placed on the plants on two different dates, but rainfall either immediately after or three days later

apparently drown all the larvae. Therefore, we were not able to evaluate feeding by armyworms for a longer period of time.

Larval mortality did indicate an interesting trend. In each of three laboratory experiments, 12.5-17.5 percent more larvae died that fed for 48 hours on transgenic *Bt* corn than on the isogenic leaf tissue. However, for those larvae that survived, there were no differences in pupal weight or days to moth emergence.

Corn earworm. No differences were noticed in the average number of larvae surviving to feed in the ears. Mortality was very high on both genotypes, with all of the larvae dying that fed on leaf tissue from the transgenic *Bt* corn. Likewise, larvae that consumed silks suffered high mortality with 20 percent more dying that ate silk from the *Bt* ears. There were no differences in either pupal weight or days to moth emergence for insects that consumed silk from either genotype.

European corn borer. Larvae that consumed transgenic *Bt* leaf tissue had a faster and higher rate of mortality than borers that ate the isogenic leaves. After feeding on transgenic *Bt* leaves for 48 hours, 32.5 percent of the corn borers died compared to only 5 percent feeding on the non*Bt* leaf tissue. Within 72 hours, mortality reached 82.5 percent and 95 percent had died at the end of two weeks for larvae that ate the transgenic *Bt* corn.

In the field study, there were noticeable reductions in the number of live borers per plant at harvest, average tunnel length per plant, and tunnels per ear shank for the transgenic *Bt* corn. Suprisingly, the yields per ear were so small between the two genotypes that no significant differences were noted. This in spite of the reduction in plant injury.

In summary, the preliminary test results suggest that the Ciba 3906 transgenic *Bt* corn does not have any effect on black cutworms and stalk borers. There is a trend for increased mortality of newly-hatched corn earworms and armyworms. Mortality of European corn borers is very high and the amount of plant injury in the field is greatly reduced, but we have yet to see an increase in yield.

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Table 1. Average responses of five caterpillar species feeding on isogenic and transgenic *Bt* corn (Ciba Seeds 3906) in Iowa, 1994. Data are preliminary.

Insect	Variable[a]	Isogenic (non B.t.)	Transgenic (B.t.)
Black cutworm	leaf injury rating	1.9	1.9
	cutting injury rating	3.4	3.2
	larval mortality	22.5%	17.5%
	pupal weight	0.20 g	0.19 g
Stalk borer	leaf injury rating	1.8	1.3
	inches of tunneling	0.41	0.40
	larval mortality	47.5%	60.0%
	pupal weight	0.42 g	0.45 g
	days to moth emergence	32.2	32.1
Armyworm	feeding scars < 2 cm	21.9	14.0
	feeding scars > 2 cm	2.0	1.0
	Experiment 1		
	larval mortality	47.5%	60.0%
	pupal weight	0.42 g	0.45 g
	days to moth emergence	32.2	32.1
	Experiment 2		
	larval mortality	27.5%	45.0%
	pupal weight	0.39 g	0.37 g
	days to moth emergence	33.6	35.0
	Experiment 3		
	larval mortality	62.5%	80.0%
	pupal weight	0.34 g	0.36 g
	days to moth emergence	38.4	38.0
Corn earworm	larvae/ear	0.78	0.73
	larval mortality-leaf tissue	80.0%	100%
	pupal weight	0.47 g	—
	larval mortality-silks	50.0%	70.0%
	pupal weight	0.43 g	0.42 g
	days to moth emergence	31.8	32.5
European corn borer	yield/ear	213 g	224 g
	borers/plant at harvest	0.29	0.18
	tunnel length/plant	8.25 cm	4.48 cm
	tunnels/ear shank	0.95	0.57
	larval mortality 48 hrs	5.0%	32.5%
	larval mortality 72 hrs	15.0%	82.5%
	larval mortality 2 wks	62.5%	95.0%

[a]/ See materials and methods for details.

Part III

FIELD TESTING OF TRANSGENIC B.T. CORN ON AN OLD CHRONIC PEST OF CORN, THE EUROPEAN CORN BORER

William B. Showers

Experimental product, MON 80200, corn seed containing HD-1 protein of Bacillus thuringiensis subsp. kurstaki was planted along with seeds of the same hybrid (MON 80200) without the B.t. kurstaki protein in a randomized complete block design with 12 experimental units. Some of the experimental units were also analyzed as a factorial arrangement. Each unit was a four row plot planted on 30" spacing by 30' length. Each plot was separated by a guard row of corn containing the HD-1 protein. Each of six replicates were separated by a 10' middle. The objective of the study was to determine the effect of this protein on Ostrinia nubilalis (Hübner), the European corn borer (ECB).

This study was conducted on the Iowa State University Woodruff Farm approximately 3 mi southwest of Ames, IA. It was part of a regional study in cooperation with Monsanto Company, University of Illinois, Iowa State University, Kansas State University, Missouri Valley Agri-Serv, Columbia, MO, University of Nebraska, Sanders Agric. Res. Serv., Steele, MO, and the Agricultural Research Service, U.S.D.A., Ames, IA and Columbia, MO.

The Iowa test was planted 14 May 1994. To avoid natural ECB first generation infestation, beginning 16 June 1994, some experimental units received weekly application of a pyrethroid (Pounce) at 8 oz/A. To insure an adequate infestation certain experimental units received 50 ECB neonate larvae per plant on 30 June and again 1 July. To simulate grower activities, 5 days later some of these experimental units received an application of Pounce at 8 oz/A or an application of granular Dipel (B.t. kurstaki) at 12 oz/1000 ft of row. With anthesis (silking and tasseling), to avoid natural ECB second generation beginning 19 July certain experimental units that had not received insecticide applications were added to the regimen and received weekly applications of Pounce at 8 oz/A. Again, to insure an adequate infestation, specific experimental units received 50 ECB neonate larvae per plant on 20 July and again 21 July. Once more to simulate potential grower activity, 5 days later some of these experimental units received an application of Pounce at 8 oz/A or an application of granular Dipel at 12 oz/1000 ft of row.

Data were collected on egg masses in the crop, shot-holes during whorl stage, stay-green plants after ear filling, entrance holes in the stalk, tunnels and larvae in the stalk and ear shank, and grain yield adjusted to 15.5% moisture. After analysis of variance the means were separated using the Ryan-Einot-Gabriel-Welsch Multiple Range Test.

The Guthrie leaf rating scale of no leaf feeding equals one and many shot-holes and portions of leaf removed equal 9 was used to determine the severity of whorl damage (first-generation). These data are presented in table 1. All of the experimental units that contained activated HD-1 protein rated near one. Non B.t. corn that was not protected by weekly pyrethroid applications rated 4.8 to 5.9. The two experimental units (3 and 7) protected during first-generation activity rated similarly to the B.t. corn.

Inches of tunneling indicated by the presence of second-generation ECB are presented in table 2. All B.t. corn experimental units had less than two inches of total tunneling per 10 plants, whereas non B.t. corn units that were not adequately protected from anthesis to dough stage of the ear ranged from 40 to 69 inches of tunneling per 10 plants. Experimental unit 2, non B.t. corn protected weekly from ECB had 14 inches of total tunnels per 10 plants. The two units (5 and 6) that were treated with Dipel and Pounce 5 days after manual infestation of ECB sustained 37 and 25 inches of total tunnels per 10 plants, respectively.

The bottom line is yield if hybrids expressing HD-1 protein of B.t. kurstaki are to be successful against the European corn borer. These data are presented in table 3. All experimental units (8-12) that contained the protein had similar yields. A significant difference occurred between most units containing the protein and units 1 and 4, non B.t. corn unprotected by insecticide. An interesting aspect of the study is that experimental unit 3, non B.t. corn protected from first-generation ECB but exposed to second-generation ECB, yielded similarly to the B.t. corn experimental units.

Table 1. Relationships of 1st-generation European corn borer larval leaf feeding on a corn hybrid expressing or not expressing the cryIA(b) gene, Ames, IA, 1994.

Experimental Units	Leaf Ratings[1] (Guthrie 1-9 Scale)
non-Bt, no treatment, 1st & 2nd infestation	5.9 a
non Bt, anthesis to dough, 1st infestation	5.7 ab
non Bt, Dipel treatment, 1st & 2nd infestation	5.2 ab
non Bt, no treatment, no infestation	5.1 ab
non Bt, Pounce treatment, 1st & 2nd infestation	4.9 b
non Bt, whorl to V15, 2nd infestation	1.3 c
non Bt, whorl to dough, no infestation	1.2 c
Bt, all	1.0 - 1.1 c

[1]Mean separations conducted using the Ryan-Einot-Gabriel-Welsch Multiple Range Test, means followed by the same letter are not significantly different at the .05 level.

Table 2. Relationships of European corn borer larval tunneling into stalk and ear shank of a corn hybrid expressing or not expressing the cryIA(b) gene, Ames, IA, 1994.

Experimental Units	Total Tunneling[1]
non-Bt, no treatment, no infestation	69.3 a
non Bt, no treatment, 1st and 2nd infestation	49.0 b
non Bt, whorl to V15, 2nd infestation	40.3 bc
non Bt, Dipel treatment, 1st & 2nd infestation	36.7 bc
non Bt, Pounce treatment, 1st & 2nd infestation	25.1 cd
non Bt, anthesis to dough, 1st infestation	14.1 de
Bt, no treatment, no infestation	1.9 e
Bt, anthesis to dough, 1st infestation	1.7 e
non Bt, whorl to dough, no infestation	1.4 e
Bt, all others	0.0 - 0.8 e

[1]Mean separations conducted using the Ryan-Einot-Gabriel-Welsch Multiple Range Test, means followed by the same letter are not significantly different at the .05 level.

Table 3. European corn borer impact on yield of a corn hybrid expressing or not expressing the cryIA(b) gene, Ames, IA 1994.

Experimental Units	Yield[1] (bu/Acre)
Bt, no treatment, no infestation	144.3 a
non Bt, whorl to dough, no infestation	143.2 a
Bt, no treatment, 1st & 2nd infestation	142.7 a
Bt, whorl to V15, 2nd infestation	142.4 a
Bt, whorl to dough, no infestation	141.5 a
non Bt, whorl to V15, 2nd infestation	141.4 a
Bt, anthesis to dough , 1st infestation	139 4 ab
non Bt, Dipel treatment, 1st & 2nd infestation	128.6 abc
non Bt, anthesis to dough, 1st infestation	127.6 abc
non Bt, Pounce treatment, 1st & 2nd infestation	127.3 bc
non Bt, no treatment, no infestation	121.3 bc
non Bt, no treament, 1st & 2nd infestation	117.0 c

[1]Mean separations conducted using the Ryan-Einot-Gabriel-Welsch Multiple Range Test, means followed by the same letter are not significantly different at the .05 level.

Part IV

THE PRESENCE OF PREDATORS OF EUROPEAN CORN BORER ON TRANSGENIC BT CORN VERSUS ISOGENIC NONBT CORN

Clinton D. Pilcher and John J. Obrycki

Introduction:

Predators can play an important part in European corn borer (ECB) population fluctuations at some locations in the midwest during some years (Sparks etal. 1966). Currently, predation cannot be depended on year after year to play a significant part in manipulating a corn borer population. An important factor in determining predator presence is the interaction between plants and natural enemies. Price (1986) refers to these different types of interactions as being intrinsic and extrinsic factors within a plant that are responsible for the attraction of natural enemies. The intrinsic and extrinsic factors of which he speaks are different types of host-plant resistance. Intrinsic factors include those proposed by Painter (1951): 1) preference and nonpreference (antixenosis), 2) antibiosis, and 3) tolerance. These factors directly affect the insect pest which may make them more susceptible to natural enemies. However, plants can also express extrinsic factors in which plants directly affect natural enemies in some way. These factors may attract natural enemies, cause them to spend more time in the habitat, or they may act as feeding stimulants.

With the production of transgenic plant technology where the plants are being altered genetically, it is not known if these changes might affect the presence of natural enemies or not. To begin to understand these relationships, it was the objective of this study to develop a predator survey and take counts of many different predator species to determine if the transgenic Bt corn plants might influence whether predator searching would take place on these plants.

Materials and Methods:

All studies are being completed on the Insectary research site, Iowa State University, Ames, IA. Transgenic and isogenic plots were planted in four row strips laying side by side. At pollen shed, beginning of second ECB generation, and during peak second generation ECB egg laying, counts were made, in accordance to Coll and Botrell (1991), for the presence of any predatory species present, as well as the life stages of these species. Three replications of 6 plants each were randomly selected in plots of transgenic corn and isogenic corn. The plants were viewed externally first and numbers were recorded. The leaf collars were parted, spiraled leaves were checked, and the silks of ears were examined. Numbers of adults, immatures, and eggs were recorded separately for each species.

Results and Discussion:

The mean numbers for each of the categories are recorded below (Table 1). There was no indication of any differences between the two types of corn. There were differences in predator numbers during the different stages of plant development and European corn borer presence. This would suggest that the presence of susceptible hosts and different plant development stages would play a more important role in determining the presence of predator species during the course of the growing season than would the use of transgenic versus isogenic corn.

Table 1: Preliminary data: Presence of different predator types on Transgenic versus Isogenic corn

	lady beetle eggs	lady beetle larvae	lady beetle pupae	12-spot lady beetle	13- spot lady beetle	H. conv. adults	Lace-wing larvae	Lace-wing eggs	Minute Pirate Bugs
Time:	Tra/Iso	Tra/Iso	Tra/Iso	Tra/Iso	Tra/Iso	Tra/Iso	Tra/Iso	Tra/Iso	Tra/Iso
pollen shed	33.7/5.7	15.7/5	-	3.7/2.3	1/.7	0/.3	-	12.3/16	11.3/15.3
2nd gen ECB	1.3/9	13.3/13.7	-	1.7/2	-	-	0/.3	10/8.7	5.7/5
Peak 2nd gen ECB	11.3/14	.3/1.7	2.3/1	2.7/4.7	-	-	-	8.3/9	14.7/12.3

* means represent number/replication. Divide by 6 to get number/plant.

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Part V

FEEDING, DEVELOPMENT AND SURVIVAL OF THE LADY BEETLE COLEOMEGILLA MACULATA (COLEOPTERA: COCCINELLIDAE) ON TRANSGENIC BT CORN POLLEN.

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Introduction

This study examined the influence of transgenic corn pollen on the survival and development of Coleomegilla maculata - the twelve-spotted lady beetle. The transgenic pollen contains a Bacillus thuringiensis subsp. kurstaki endotoxin protein that is toxic to certain Lepidopterans (Koziel et al. 1993). The twelve-spotted lady beetle is found in corn fields throughout the North Central and Northeastern United States (Gordon 1985). This predatory species feeds on corn leaf aphids, European corn borer eggs, and corn pollen (Andow 1990, Hodek 1973). The twelve-spotted lady-beetle is one of the few predators found in corn that can complete its development on corn pollen (Hodek et al. 1978, Smith 1960, 1965). These features along with its potential exposure to transgenic corn pollen, make this beetle a highly suitable species to examine for non-target effects. The objective of this study was to determine the effects of transgenic corn pollen containing a Bt protein on the feeding behavior and survival of the twelve-spotted lady beetle.

Materials and Methods

Adult beetles were collected in the Ames, IA area during the fall and winter of 1993-94. Pairs of field collected adults were maintained at a photoperiod of 16:8 (L:D), 24 ± 1 C, R.H. 30 to 50 %, and provided with a standard diet of water, a Wheast[u] (Qualcepts Nutrients, Minneapolis, Minn) -honey [1:1] mixture, and a daily supply of aphids green peach aphids and pea aphids. Egg masses from 13 first laboratory generation females were collected and placed at 26 C, L:D 16:8. Eggs hatched within 3 days; individual first instars were then placed in vials on one of the three diets - transgenic Bt pollen, isogenic non-Bt pollen and pea aphids.

Three pea aphids were provided daily to each larva in the aphid control group. The two types of corn pollen were weighed separately in glass shell vials; and individual larvae were transferred to these vials. The first instars were provided 0.015 g of pollen. The third instars were provided 0.021 g of pollen for three days; each fourth stage larva was provided with 0.030 g of pollen, until pupation.

Observations of feeding behavior of larval stages were made for each diet treatment. At 26 C, first instars will starve within 24 hrs. if they do not feed. Through these observations it was determined if first and fourth instar larvae were consuming pollen.

Results & Discussion

Fifteen first instar larvae exposed to each diet were observed under the dissecting microscope for two minute observation periods to determine if the larvae were feeding on pollen. Sixty percent of the 15 larvae exposed to isogenic pollen were observed to be feeding, whereas 53 % [8 of 15] of first instars were feeding on transgenic pollen. By comparison, 8 of 15 larvae [53 %] were observed feeding upon pea aphids.

Two minute observations were made of 28 fourth instar larvae being fed isogenic pollen and 23 larvae being fed transgenic pollen. Six of the 28 larvae [21%] were feeding on the isogenic pollen; 4 of the 23 larvae [17%] were feeding on the transgenic pollen. These observations demonstrate that first and fourth instar larvae consumed isogenic or transgenic pollen. From these brief observations we conclude that there appears to be no difference in the acceptance of either pollen as a food source by the larvae of the twelve-spotted lady beetle.

The survival of larvae on pea aphids was higher than the survival on either type of pollen. Survival on the aphid diet was expected to be greater than 80%; based upon a previous studies (Obrycki & Tauber 1978). There was no difference in survival on the two types of pollen. When reared on either pollen, death of larvae occurred primarily after the third instar. This pattern of mortality, during the fourth instar and pupal stage, does not indicate that the transgenic pollen had a toxic effect on larval stages of the twelve-spotted lady beetle.

The survival of larvae reared on isogenic and transgenic pollen was lower than expected.

Previous studies have reported variable survival of twelve-spotted lady beetles reared on corn pollen, ranging from 15 % to < 66 % (Table 1). In the field, these beetles typically feed on more than one type of prey or non-insect prey items, e.g., pollen and fungi (Hodek 1973, Smith 1965). A laboratory study using a combination of aphid prey and corn pollen would more closely mimic field conditions.

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Table 1. Summary of previous studies of Twelve-spotted lady beetle development and survival on corn pollen.

Developmental time [days]	**Survival [%]**
1st to Adult 23.9 ± 1.6	Not stated, but significantly <66%
1st to Adult 26.0 ± .3	15%
F_1 1st to Adult 22.9 ± 1.9	52%
F_2 1st to Adult 26.0 ± 1.3	50%

* The experiments were at a rearing temperature of 22C, 65% RH
** The pollen in the first study was dried at 20C, sieved, and stored at 4C
***Above studies completed by Smith (1960, 1965)

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